Stress-Related Biomarkers of Relapse Vulnerability in Addictive Disorders.

December 3, 2008

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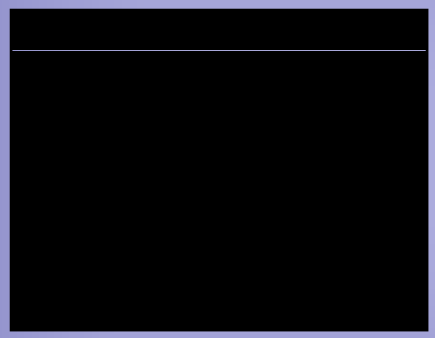
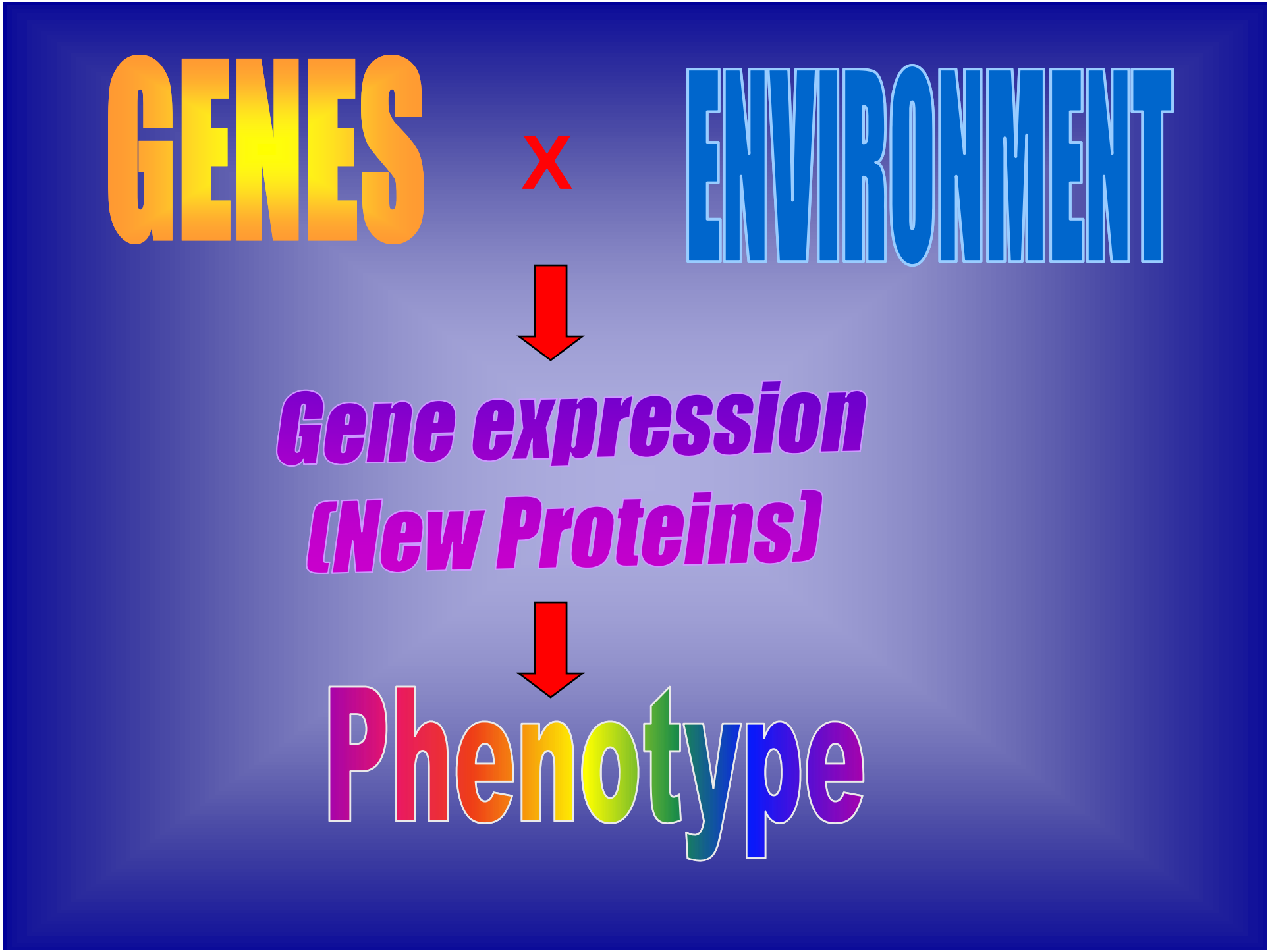
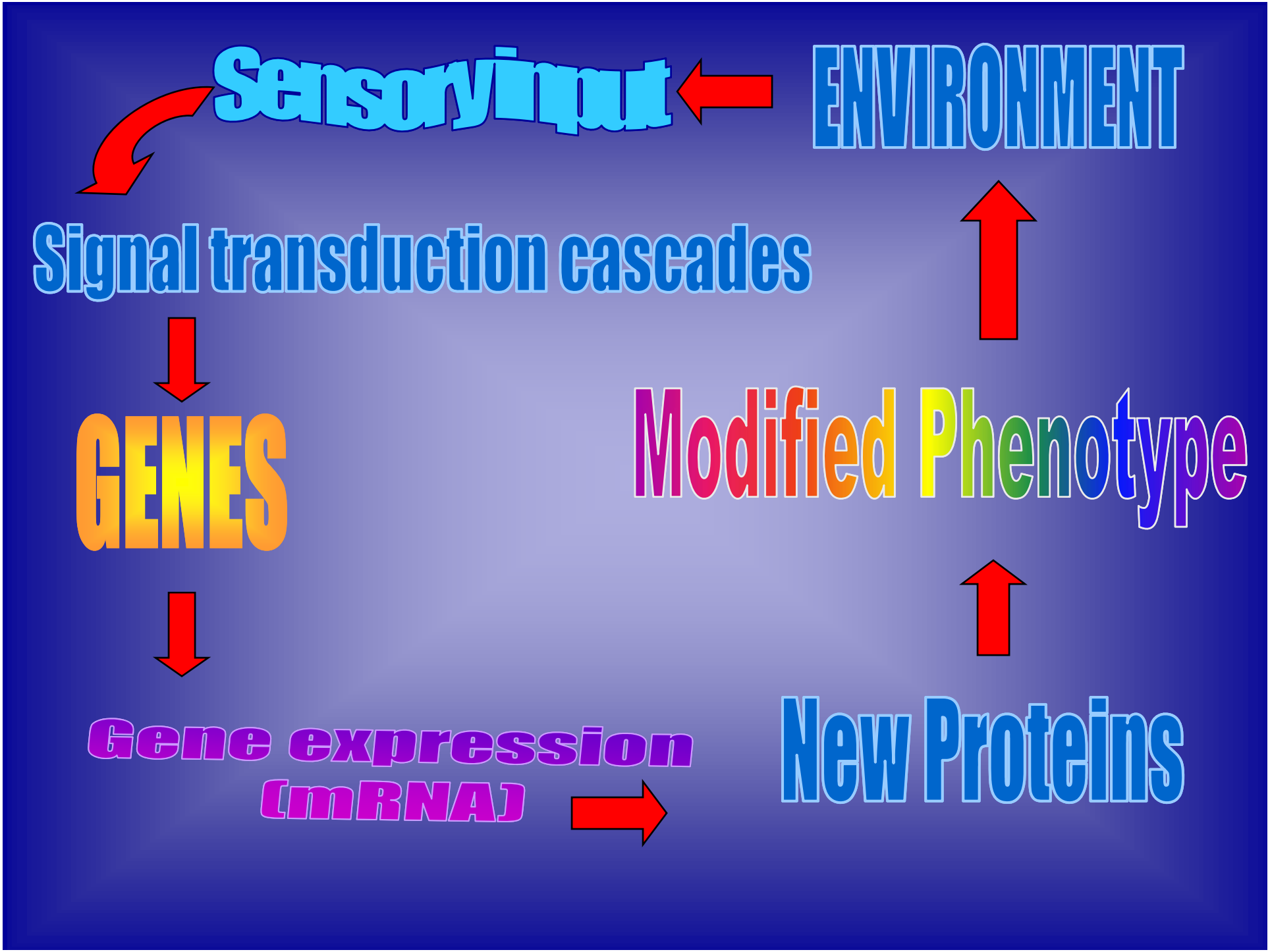
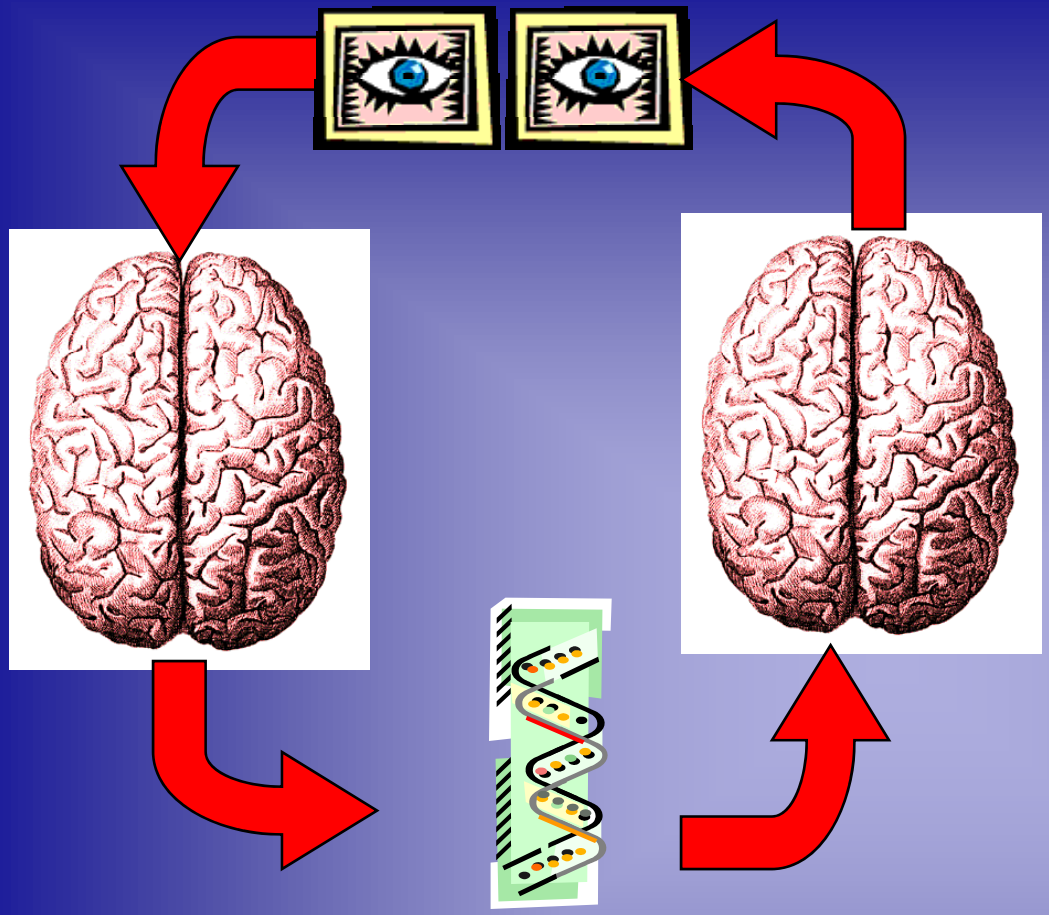
GENES x ENVIRONMENT

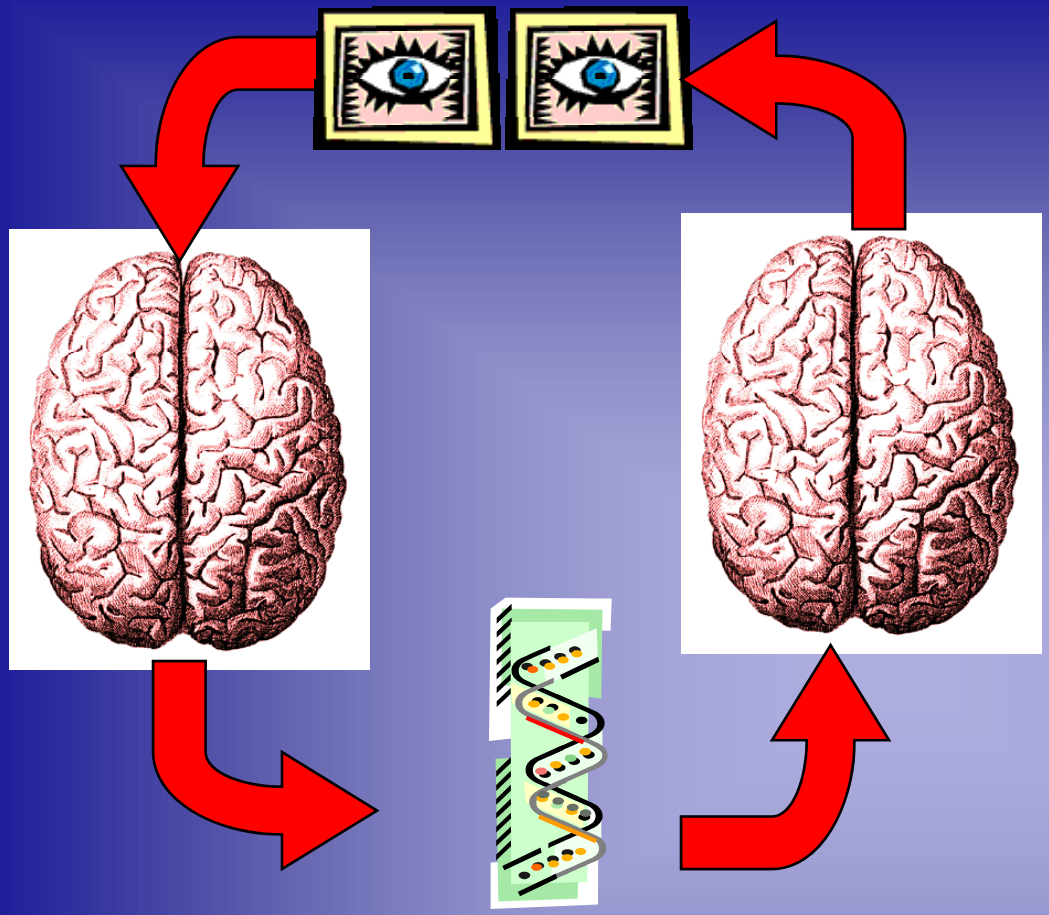


Phenotype


GENES
x
ENVIRONMENT
Gene expression
(New Proteins)
Phenotype


Sensory input
ENVIRONMENT
Signal transduction cascades
GENES
Modified Phenotype
Gene expression (mRNA)
New Proteins





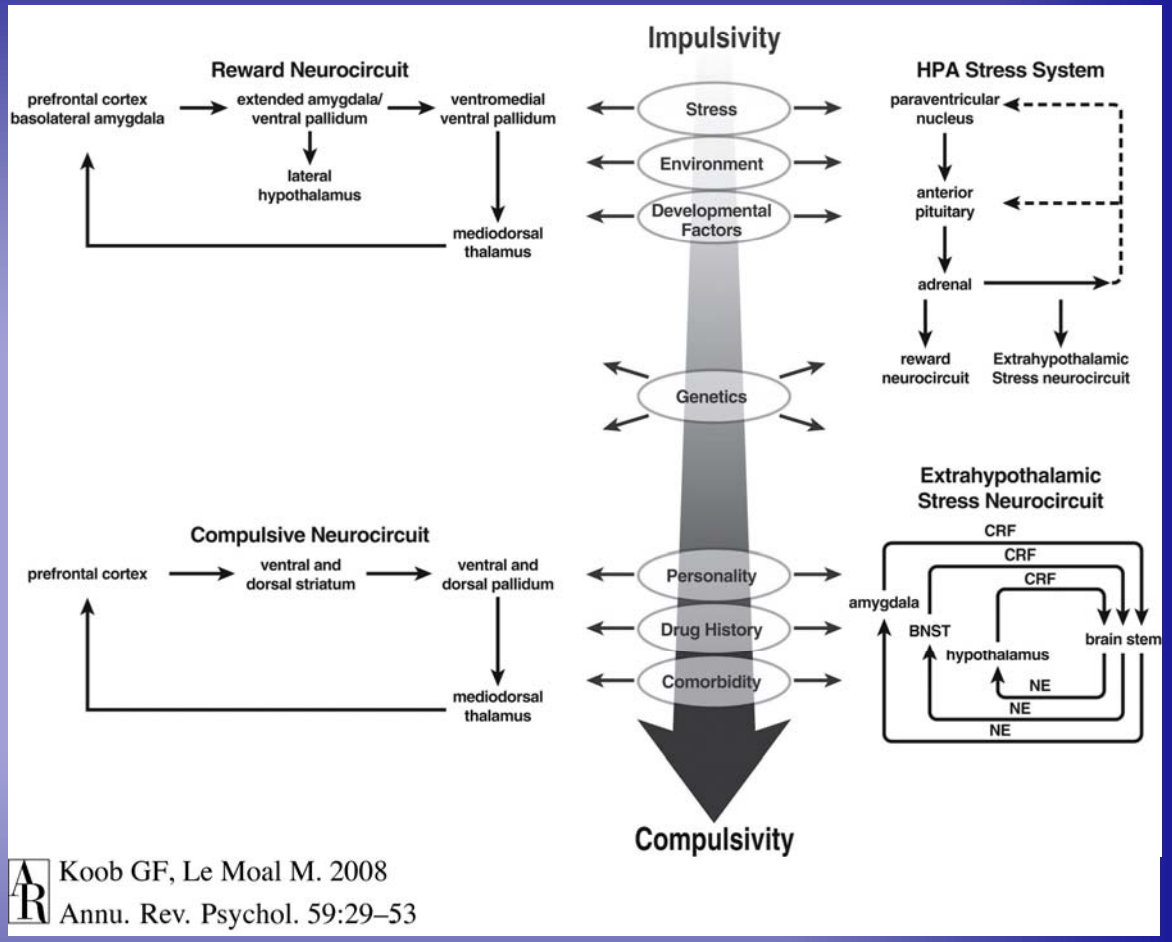
Problem:

With current techniques there is no way to measure changes in gene expression in the brains of living subjects.

Blood as a surrogate tissue to identify gene-expression changes related to Psychiatric illness including Addiction

Stress and Addiction:

Stress plays a central role in both the development of addiction and the risk for subsequent relapse to chronic drug taking after addicted individuals have achieved an initial period of sobriety.



Koob GF, Le Moal M. 2008
Annu. Rev. Psychol. 59:29–53

Affects of stress on alcohol craving can be modeled by exposure to personalized scripts describing drug or stress-related situations in a laboratory setting.

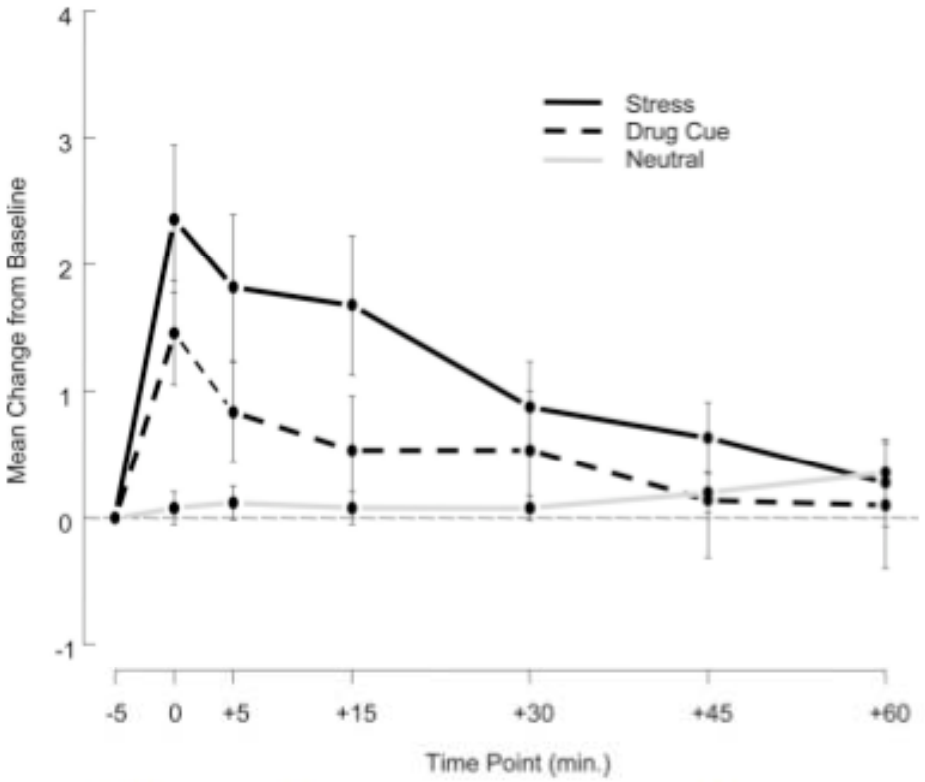


Fig. 1. Alcohol craving after stress and other imagery conditions. Shown is the mean change from baseline in alcohol craving after exposure to stress (S), drug/alcohol cue (D), and neutral relaxing (N) imagery conditions on separate days in 25 cocaine-dependent alcoholics [condition main effect: $F(2,408) = 18.1$, $p < 0.0001$; S > DC > N] immediately after imagery exposure (0 time point) and up to 60 min after imagery exposure [time point main effect: $F(5, 408) = 4.5$, $p < 0.0005$].

Breese, *et al.* (2005). Stress enhancement of craving during sobriety: a risk for relapse. Alcohol Clin Exp Res *29*, 185-195.

Cue induced craving in the laboratory predicts alcohol use in the "real world".

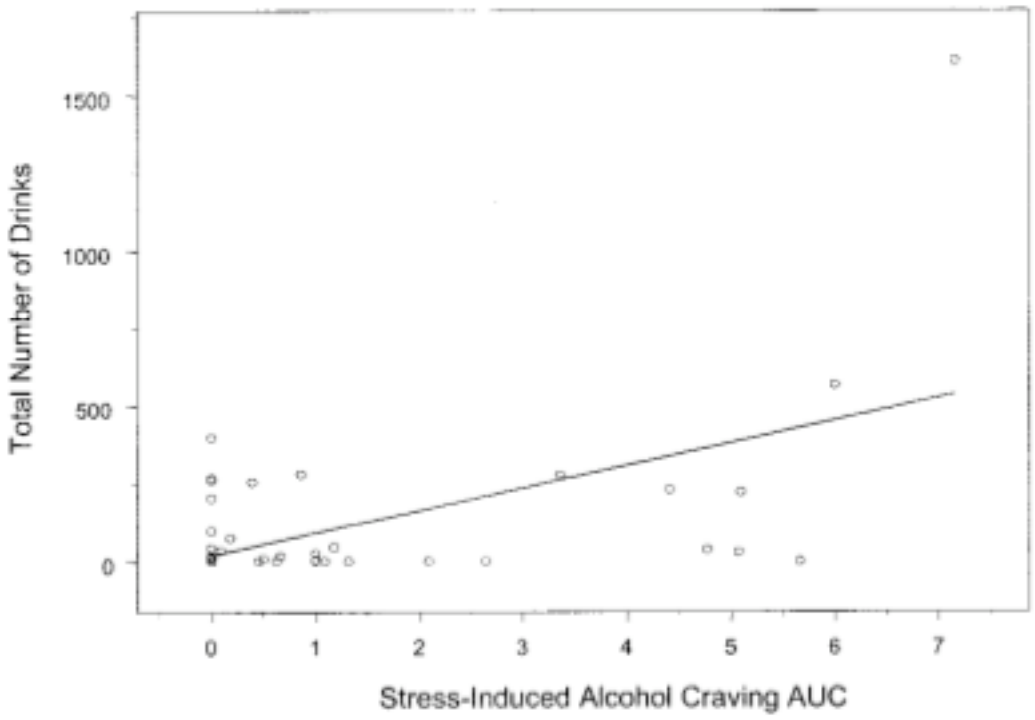
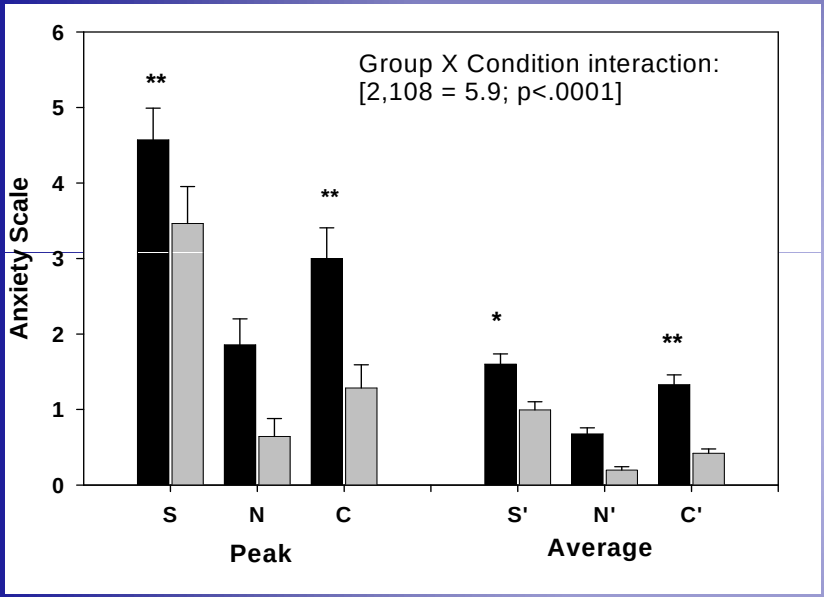


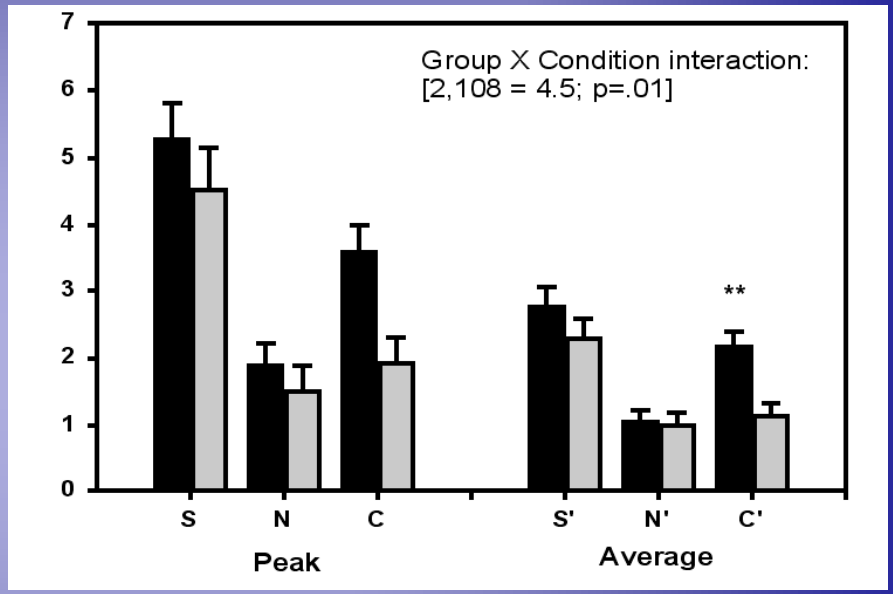
Fig. 2. Relation of stress-induced craving and drinks consumed. Scatterplot for stress-induced alcohol craving area under the curve (AUC) response during the inpatient laboratory session and total number of drinks consumed in the 90 days after discharge from inpatient drug treatment ($R^2 = 0.30$, $r = 0.55$, $p < 0.0001$) in 49 cocaine- and alcohol-abusing patients. Alcohol craving in response to drug/alcohol cues or to neutral relaxing imagery exposure was not associated with drinking after discharge.

Breese, *et al.* (2005). Stress enhancement of craving during sobriety: a risk for relapse. Alcohol Clin Exp Res *29*, 185-195.

Addicted individuals show altered subjective responses to drug and stress related imagery



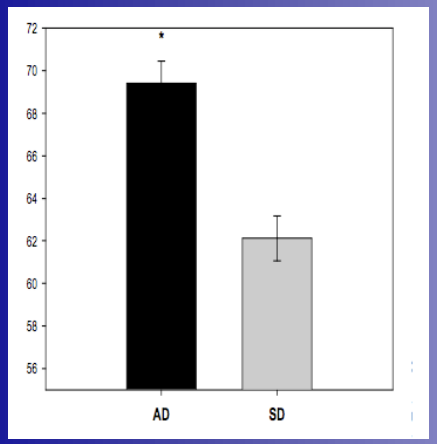
Peak and Average anxiety ratings following exposure to Stress (S), Neutral (N), and Drug Cue (C) related imagery in Alcoholics (dark bars) and Controls (light bars)



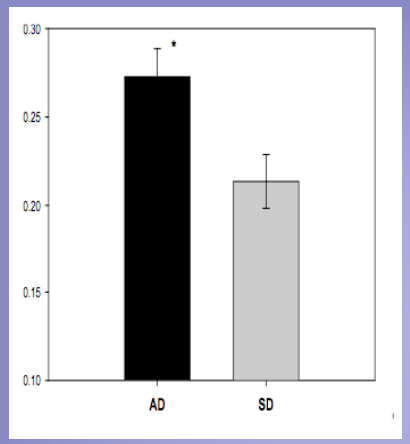
Peak and Average ratings of behavioral distress following exposure to Stress (S), Neutral (N), and Drug Cue (C) related imagery in Alcoholics (dark bars) and Controls (light bars)

Addicted individuals show altered physiological responses to drug and stress related imagery

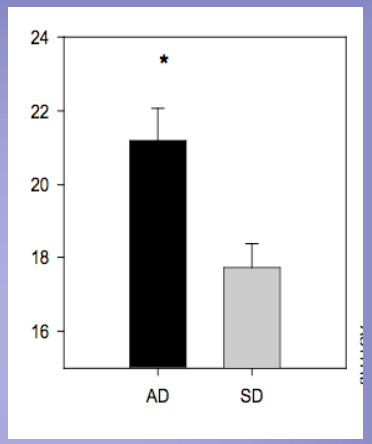
Basal HR



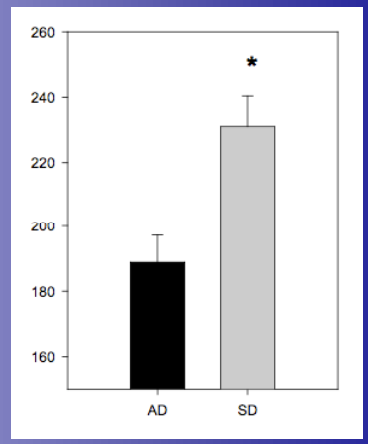
Basal Cortisol



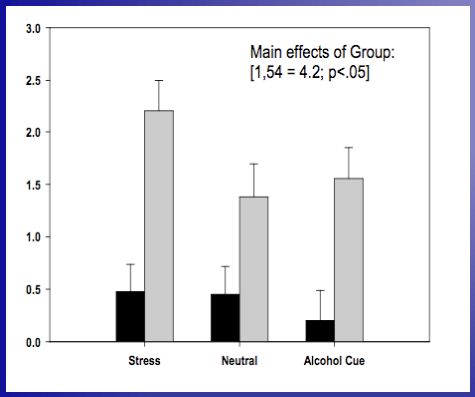
Basal ACTH



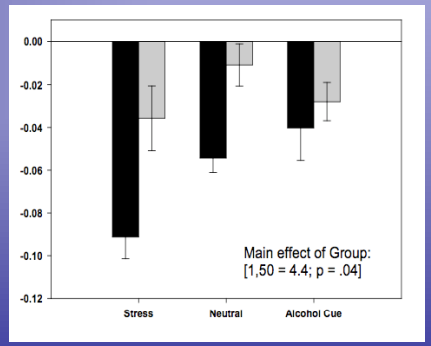
Basal NE



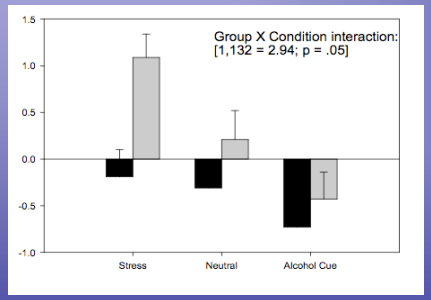
HR Response



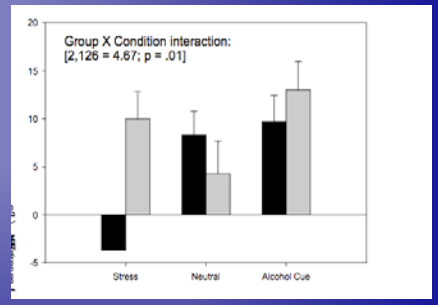
Cortisol Response



ACTH Response



NE Response



- These findings indicate the chronic drug abuse is associated with alterations in basal stress tone (high HR, ACTH and cortisol, low NE) and enhanced stress and cue-related craving, anxiety, behavioral distress and negative emotions with a suppressed heart rate, cortisol, ACTH, and NE response.
- Changes in the subjective and physiological response to stress are believed to occur as a result of changes in gene-expression following chronic exposure to drugs of abuse. (Koob, 2003; Mailliard and Diamond, 2004; Rodd et al., 2007; Sanna et al., 2002).
- However, to date there are no studies, to our knowledge, that have systematically examined stress-related changes in protein expression in alcohol-dependent subjects in vivo.

Long term goal:

To characterize the alterations in gene and protein expression that both result from and contribute to long term use of addictive substances in order to:

(1) Identify biomarkers that may be associated with increased risk for relapse. Such biomarkers would allow clinicians to target high-risk individuals for more intensive clinical interventions and may serve as surrogate endpoints for future studies assessing the effects of specific treatments.

(2) Identify alterations in regulatory networks that will help us to better understand the nature of relapse vulnerability in addiction and develop of novel treatments for addiction.

Pilot study:

Differences in serum protein levels between Alcohol Dependent Subjects (n=30), Cocaine dependent subjects (n=30) and Healthy Controls (n=30)

Objective: To determine whether serum protein expression profiles differ significantly between these three groups and, if so, which genes and pathways were involved.

Alcohol and Cocaine Dependent:

- Male or Female, age between 18 and 65.
- DSM-IV-TR Diagnosis of Alcohol or Cocaine Dependence (based on clinical exam by Board Certified Psychiatrist and confirmed by SCID and positive urine toxicology for cocaine dependent subjects).
- Do not meet criteria for dependence on other psychoactive substances, other than nicotine
- Do not meet criteria for any other DSM-IV axis I psychiatric disorder

Healthy Controls:

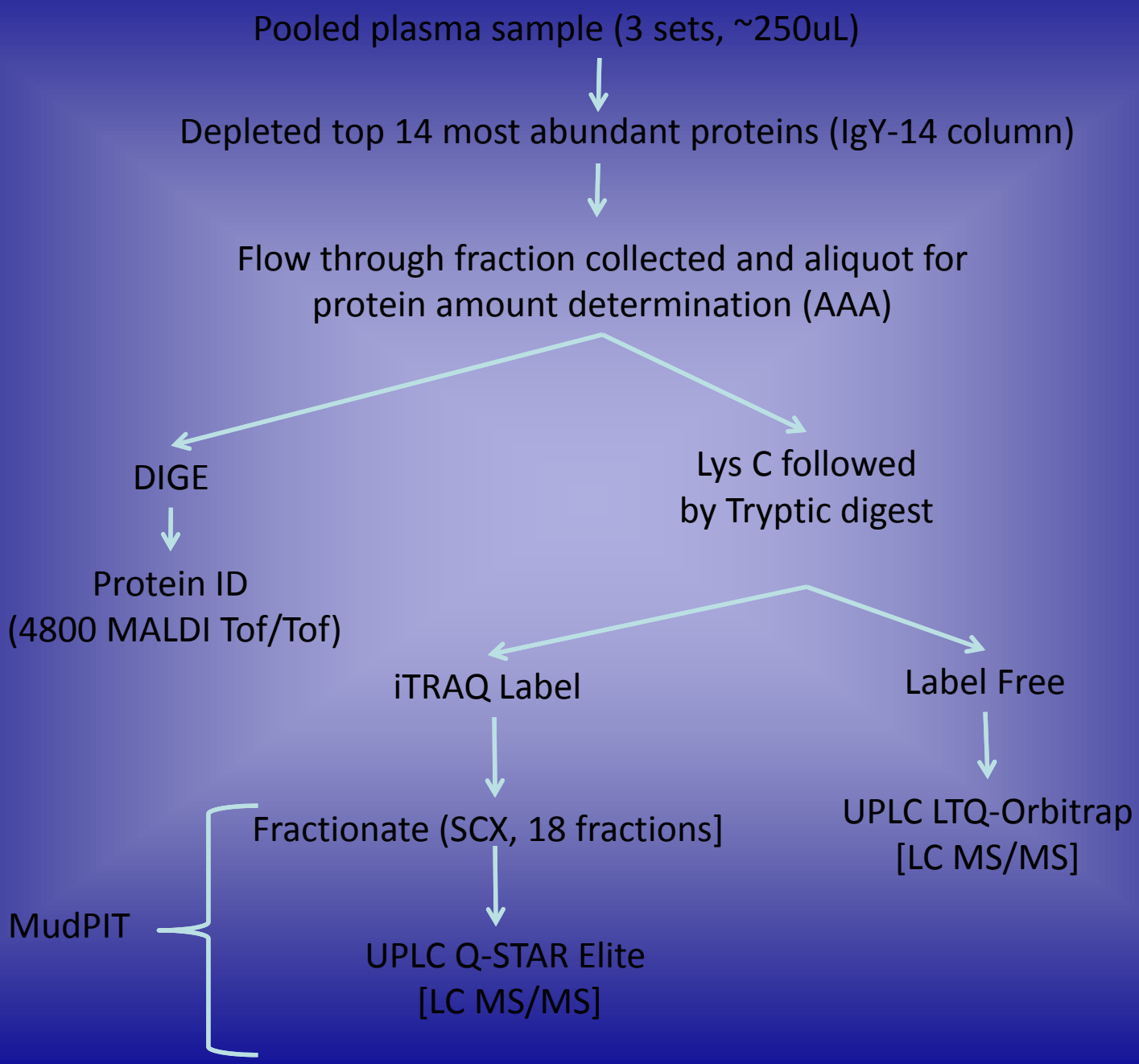
- Male or Female, age between 18 and 65.
- Drink up to 25 drinks or less per month
- Do NOT meet criteria for current or lifetime abuse or dependence for alcohol or any other illicit drug
- Do not meet criteria for any other DSM-IV axis I psychiatric disorder

Samples used in parallel iTRAQ, Label Free, and DIGE quantitation

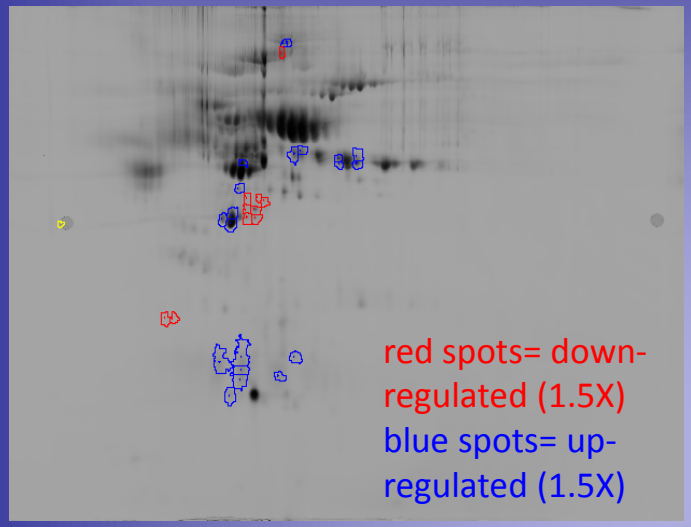
SID	Tube # June 1, 2007	Study	1=Male 2=Female	Cauc	African Amer	Latino	Asian	Native Amer	Age
A265	**AD012**	AD	1	1	0	0	0	0	37
A337	**AD015**	AD	1	1	0	0	0	0	43
A1153	**AD049**	AD	1	1	0	0	0	0	48
A1177	**AD050**	AD	1	1	0	0	0	0	41
A769	**AD033**	AD	1	0	1	0	0	0	35
A1225	**AD052**	AD	1	0	1	0	0	0	46
481	**COC086**	COC	1	1	0	0	0	0	35
505	**COC087**	COC	1	1	0	0	0	0	38
793	**COC099**	COC	1	1	0	0	0	0	47
1105	**COC111**	COC	1	1	0	0	0	0	42
961	**COC001**	COC	1	0	1	0	0	0	41
865	**COC102**	COC	1	0	1	0	0	0	47
H625	**HC027**	HC	1	1	0	0	0	0	40
H193	**HC009**	HC	1	1	0	0	0	0	38
H121	**HC006**	HC	1	1	0	0	0	0	43
H1009	**HC043**	HC	1	1	0	0	0	0	46
H529	**HC023**	HC	1	0	1	0	0	0	50
H409	**HC018**	HC	1	0	1	0	0	0	47

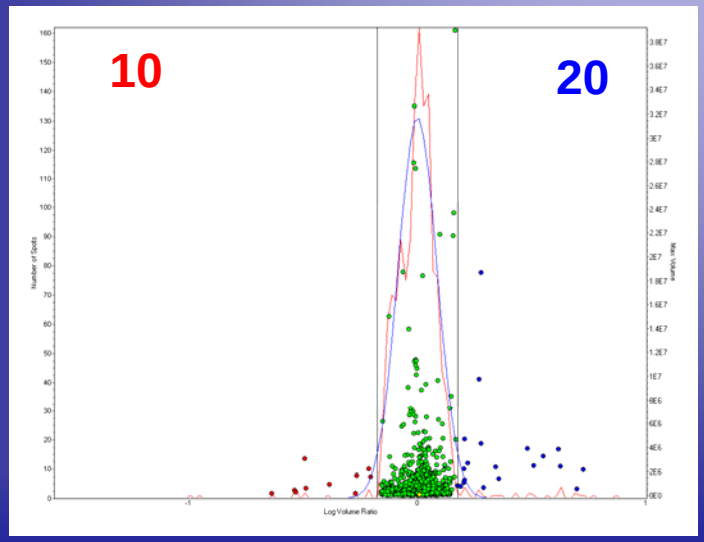
- All Male ages 35-50
- 6 samples from each set are pooled (4 Cauc + 2 African American)
- All samples collected and handled uniformly, stored at -80^{o}C until analysis

Methodology

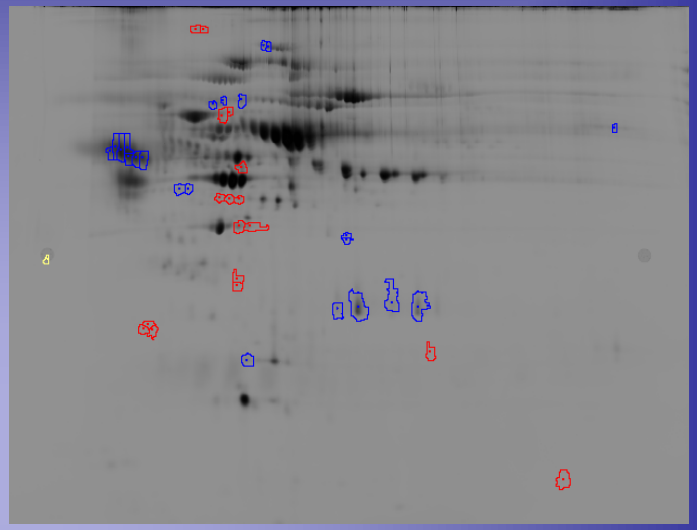


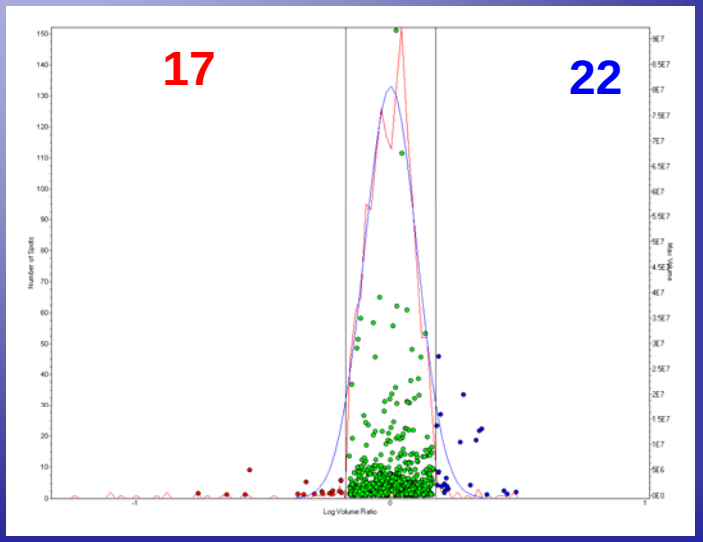
DIGE Gel 1086, spot distribution (Cy5:Cy3 Cocaine:Healthy)





DIGE Gel 1082, spot distribution (Cy5:Cy3 Alcohol:Healthy)

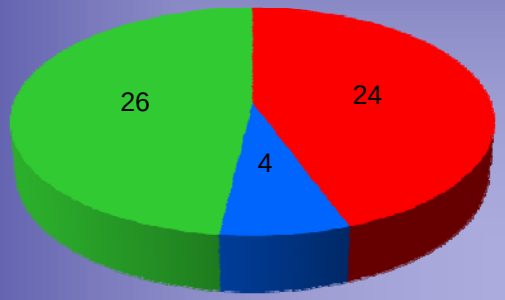




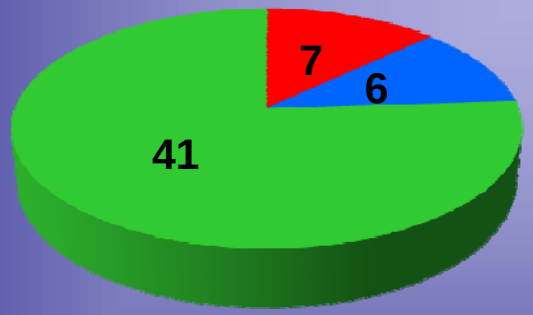
Label Free

(LTQ Orbitrap MS)

Healthy vs. Cocaine



Healthy vs. Alcohol



Label Free Down regulated

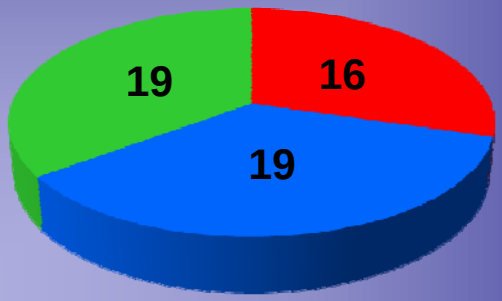
Label Free Up regulated

Label Free No change

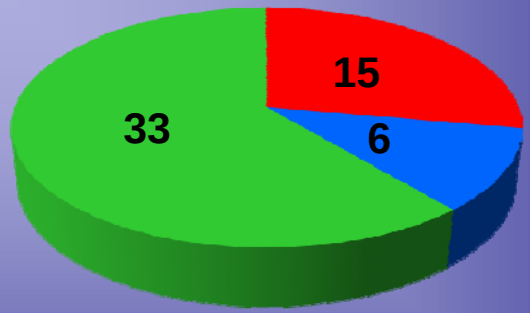
iTRAQ

(Q-STAR Elite MS)

Healthy vs. Cocaine



Healthy vs. Alcohol



iTRAQ Down regulated

iTRAQ Up regulated

iTRAQ No change

**Top 54 protein ID scores from each Techniques

Summary:

•Serum protein expression profiles were compared among Alcohol and Cocaine dependent subjects and healthy controls using three different methodologies (DIGE, iTRAQ, and Label-free [LC MS/MS].

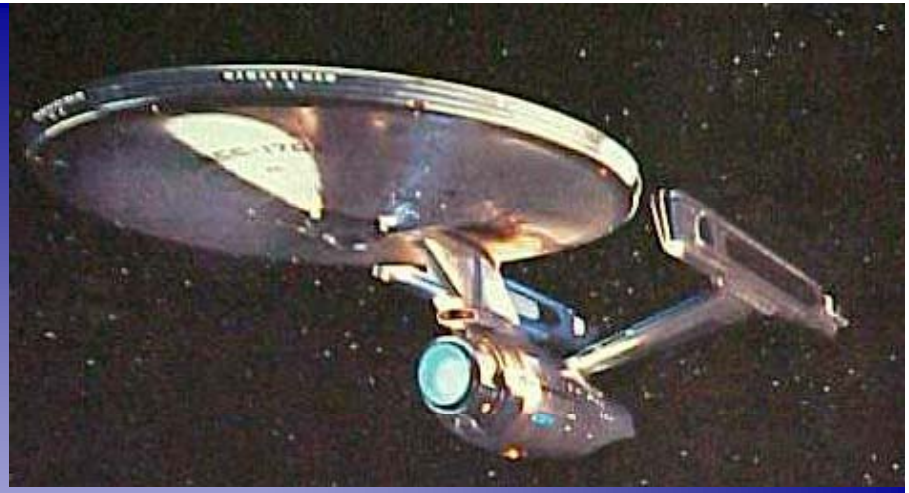
•Of these Label free LC MS/MS seems the most suited for high throughput analysis of samples collected from individual subjects under different experimental conditions.

•Both Alcohol and Cocaine dependent subjects showed numerous differences in peripheral blood protein profiles compared to healthy controls.

• Abnormal expression of stress-related proteins may underlie the heightened response to stress observed in both alcohol and cocaine dependent subjects.

• Individual differences among drug-dependent subjects may relate to individual differences in response to treatment, risk for relapse, and co-morbid disorders (e.g. depression).

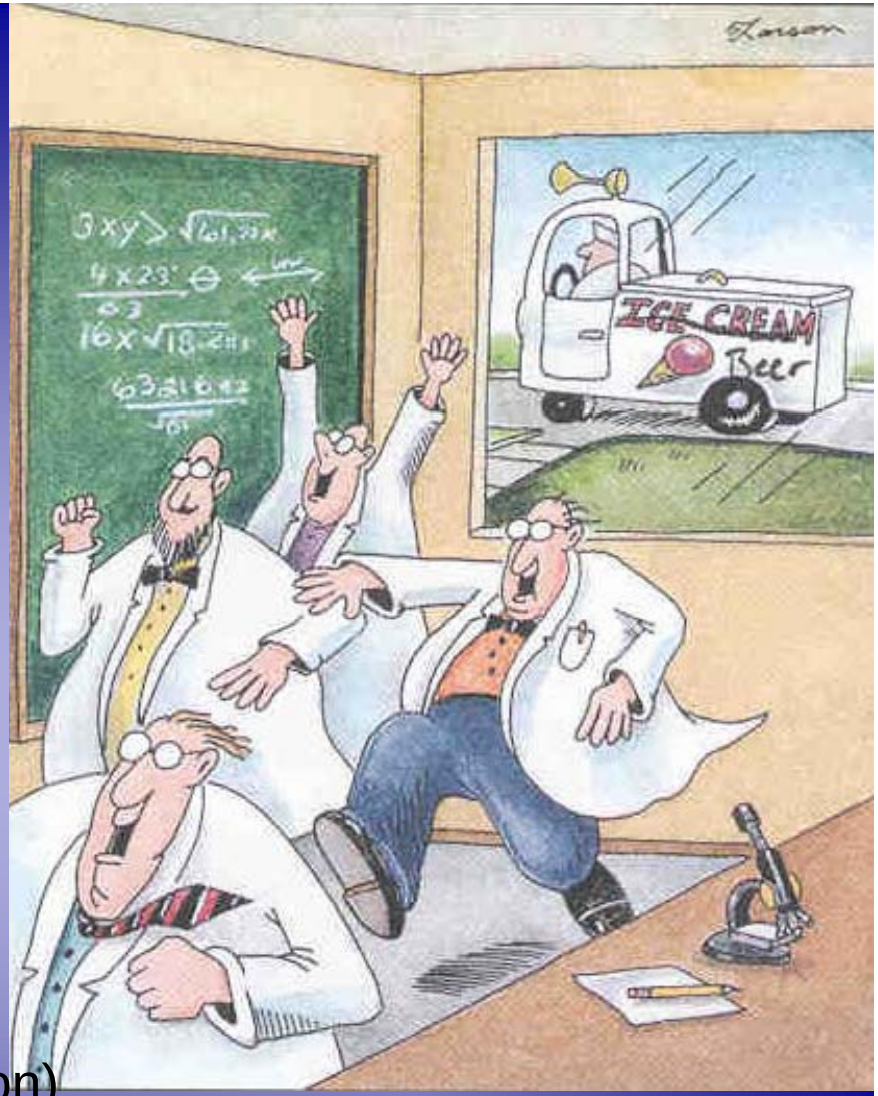
Future Directions!



- Moving from group differences to individual variations.
- Moving from descriptive to predictive.

New study:

- Assess the relationship between candidate biomarkers and psychological, physiological, and biochemical indices of stress during exposure to stress and drug cues in addicted samples (cocaine, alcohol).
- Validate candidate biomakers of sress-related vulnerability in a new cohort of addicted subjects (cocaine, alcohol; n=30/group) as compared to healthy non-addicted control volunteers (n=30).
- Determine whether specific treatment interventions targeting stress-induced vulnerability to relapse affect expression of candidate

NIH/ NIDA Funding
K12 DA-00167
RO1-AA11389
RO1-AA13892
RO1-DA18219
KO2-DA17232
P5O- DA16556

Collaborators
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Myron Crawford (AAA)
Kathy Kanarek (CNRU)
Jean Kanyo (Protein ID, MALDI Tof/Tof)
Adam Hong (CNRU)
TuKiet (Tu) Lam (FT-ICR)
Janine Leffert (9th Floor 300 George)
Mary LoPresti (Protein ID, Plasma depletion)
Rajita Sinha (CNRU)
Kathy Stone (Protein ID, LTQ-Orbitrap)
Jennifer Sutton (Thermo Scientific, SIEVE/label free)
Katherin Wilczak-Havill (Protein Profiling, iTRAQ)